



RESULTS RECIPIENT
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 Report Date: 09/25/2017

MALE
DONOR 10177
 DOB: ██████████
 Ethnicity: Northern European
 Sample Type: EDTA Blood
 Date of Collection: 09/16/2017
 Date Received: 09/18/2017
 Date Tested: 09/25/2017
 Barcode: 11004212142879
 Indication: Egg or sperm donor

FEMALE
 N/A

Foresight™ Carrier Screen

NEGATIVE

ABOUT THIS TEST

The **Counsyl Foresight Carrier Screen** utilizes sequencing, maximizing coverage across all DNA regions tested, to help you learn about your chance to have a child with a genetic disease.

RESULTS SUMMARY

Risk Details	DONOR 10177	Partner
Panel Information	Foresight Carrier Screen Universal Panel Minus X-Linked (102 conditions tested)	N/A
All conditions tested A complete list of all conditions tested can be found on page 4.	<input checked="" type="checkbox"/> NEGATIVE No disease-causing mutations were detected.	N/A

CLINICAL NOTES

- None

NEXT STEPS

- If necessary, patients can discuss residual risks with their physician or a genetic counselor.

Counsyl has renamed its products effective July 19, 2017. The Family Prep Screen is now the Foresight Carrier Screen. The new names now appear on all communications from Counsyl. If you have any questions, please contact Counsyl directly.

Methods and Limitations

DONOR 10177 [Foresight Carrier Screen]: sequencing with copy number analysis, spinal muscular atrophy, and analysis of homologous regions.

Sequencing with copy number analysis

High-throughput sequencing and read depth-based copy number analysis are used to analyze the listed exons, as well as selected intergenic and intronic regions, of the genes in the Conditions Tested section of the report. The region of interest (ROI) of the test comprises these regions, in addition to the 20 intronic bases flanking each exon. In a minority of cases where genomic features (e.g., long homopolymers) compromise calling fidelity, the affected intronic bases are not included in the ROI. The ROI is sequenced to high coverage and the sequences are compared to standards and references of normal variation. More than 99% of all bases in the ROI are sequenced at greater than the minimum read depth. Mutations may not be detected in areas of lower sequence coverage. Small insertions and deletions may not be as accurately determined as single nucleotide variants. Genes that have closely related pseudogenes may be addressed by a different method. *CFTR* and *DMD* testing includes analysis for both large (exon-level) deletions and duplications with an average sensitivity of 99%, while other genes are only analyzed for large deletions with a sensitivity of >75%. However, the sensitivity may be higher for selected founder deletions. If *GJB2* is tested, two large upstream deletions which overlap *GJB6* and affect the expression of *GJB2*, *del(GJB6-D13S1830)* and *del(GJB6-D13S1854)*, are also analyzed. Mosaicism or somatic variants present at low levels may not be detected. If detected, these may not be reported.

Detection rates are determined by using literature to estimate the fraction of disease alleles, weighted by frequency, that the methodology is unable to detect. Detection rates only account for analytical sensitivity and certain variants that have been previously described in the literature may not be reported if there is insufficient evidence for pathogenicity. Detection rates do not account for the disease-specific rates of de novo mutations.

All variants that are a recognized cause of the disease will be reported. In addition, variants that have not previously been established as a recognized cause of disease may be identified. In these cases, only variants classified as "likely" pathogenic are reported. Likely pathogenic variants are described elsewhere in the report as "likely to have a negative impact on gene function". Likely pathogenic variants are evaluated and classified by assessing the nature of the variant and reviewing reports of allele frequencies in cases and controls, functional studies, variant annotation and effect prediction, and segregation studies. Exon level duplications are assumed to be in tandem and are classified according to their predicted effect on the reading frame. Benign variants, variants of uncertain significance, and variants not directly associated with the intended disease phenotype are not reported. Curation summaries of reported variants are available upon request.

Spinal muscular atrophy

Targeted copy number analysis is used to determine the copy number of exon 7 of the *SMN1* gene relative to other genes. Other mutations may interfere with this analysis. Some individuals with two copies of *SMN1* are carriers with two *SMN1* genes on one chromosome and a *SMN1* deletion on the other chromosome. This is more likely in individuals who have 2 copies of the *SMN1* gene and are positive for the g.27134T>G SNP, which affects the reported residual risk; Ashkenazi Jewish or Asian patients with this genotype have a high post-test likelihood of being carriers for SMA and are reported as carriers. The g.27134T>G SNP is only reported in individuals who have 2 copies of *SMN1*.

Analysis of homologous regions

A combination of high-throughput sequencing, read depth-based copy number analysis, and targeted genotyping is used to determine the number of functional gene copies and/or the presence of selected loss of function mutations in certain genes that have homology to other regions. The precise breakpoints of large deletions in these genes cannot be determined, but are estimated from copy number analysis. High numbers of pseudogene copies may interfere with this analysis.

If *CYP21A2* is tested, patients who have one or more additional copies of the *CYP21A2* gene and a loss of function mutation may not actually be a carrier of 21-hydroxylase-deficient congenital adrenal hyperplasia (CAH). Because the true incidence of non-classic CAH is unknown, the residual carrier and reproductive risk numbers on the report are only based on published incidences for classic CAH. However, the published prevalence of non-classic CAH is highest in individuals of Ashkenazi Jewish, Hispanic, Italian, and Yugoslav descent. Therefore, the residual and reproductive risks are likely an underestimate of overall chances for 21-hydroxylase-deficient CAH, especially in the aforementioned populations, as they do not account for non-classic CAH. If *HBA1/HBA2* are tested, some individuals with four alpha globin genes may be carriers, with three genes on one chromosome and a deletion on the other chromosome. This and similar, but rare, carrier states, where complementary changes exist in both the gene and a pseudogene, may not be detected by the assay.

Limitations

In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, bone marrow transplantation, blood transfusions and technical errors. This test is designed to detect and report germline alterations. While somatic variants present at low levels may be detected, these may not be reported. If more than one variant is detected in a gene, additional studies may be necessary to determine if those variants lie on the same chromosome or different chromosomes. The test does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78. Obstet. Gynecol. 2007;109:229-37*).

This test was developed and its performance characteristics determined by Counsyl, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's evaluation. CLIA Number: **#05D1102604**.

LAB DIRECTORS



H. Peter Kang, MD, MS, FCAP

Conditions Tested

21-hydroxylase-deficient Congenital Adrenal Hyperplasia - Gene: CYP21A2. Autosomal Recessive. Analysis of Homologous Regions. **Variants (13):** CYP21A2 deletion, CYP21A2 duplication, CYP21A2 triplication, G111Vfs*21, I173N, L308FfsX6, P31L, Q319*, Q319*+CYP21A2dup, R357W, V281L, [I237N;V238E;M240K], c.293-13C>G. **Detection Rate:** Northern European 96%.

ABCC8-related Hyperinsulinism - Gene: ABCC8. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000352:1-39. **Detection Rate:** Northern European >99%.

Alkaptonuria - Gene: HGD. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000187:1-14. **Detection Rate:** Northern European >99%.

Alpha Thalassemia - Genes: HBA1, HBA2. Autosomal Recessive. Analysis of Homologous Regions. **Variants (13):** -(alpha)20.5, --BRIT, --MEDI, --MEDII, --SEA, --THAI or --FIL, -alpha3.7, -alpha4.2, HBA1+HBA2 deletion, Hb Constant Spring, anti3.7, anti4.2, del HS-40. **Detection Rate:** Unknown due to rarity of disease.

Alpha-1 Antitrypsin Deficiency - Gene: SERPINA1. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000295:2-5. **Detection Rate:** Northern European >99%.

Alpha-mannosidosis - Gene: MAN2B1. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000528:1-23. **Detection Rate:** Northern European >99%.

Alpha-sarcoglycanopathy - Gene: SGCA. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000023:1-9. **Detection Rate:** Northern European >99%.

Andermann Syndrome - Gene: SLC12A6. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_133647:1-25. **Detection Rate:** Northern European >99%.

ARSACS - Gene: SACS. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_014363:2-10. **Detection Rate:** Northern European 99%.

Aspartylglycosaminuria - Gene: AGA. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000027:1-9. **Detection Rate:** Northern European >99%.

Ataxia with Vitamin E Deficiency - Gene: TTPA. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000370:1-5. **Detection Rate:** Northern European >99%.

Ataxia-telangiectasia - Gene: ATM. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000051:2-63. **Detection Rate:** Northern European 98%.

Bardet-Biedl Syndrome, BBS1-related - Gene: BBS1. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_024649:1-17. **Detection Rate:** Northern European >99%.

Bardet-Biedl Syndrome, BBS10-related - Gene: BBS10. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_024685:1-2. **Detection Rate:** Northern European >99%.

Beta-sarcoglycanopathy - Gene: SGCB. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000232:1-6. **Detection Rate:** Northern European >99%.

Biotinidase Deficiency - Gene: BTD. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000060:1-4. **Detection Rate:** Northern European >99%.

Bloom Syndrome - Gene: BLM. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000057:2-22. **Detection Rate:** Northern European >99%.

Canavan Disease - Gene: ASPA. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000049:1-6. **Detection Rate:** Northern European 98%.

Carnitine Palmitoyltransferase IA Deficiency - Gene: CPT1A. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_001876:2-19. **Detection Rate:** Northern European >99%.

Carnitine Palmitoyltransferase II Deficiency - Gene: CPT2. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000098:1-5. **Detection Rate:** Northern European >99%.

Cartilage-hair Hypoplasia - Gene: RMRP. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exon:** NR_003051:1. **Detection Rate:** Northern European >99%.

Citrullinemia Type 1 - Gene: ASS1. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000050:3-16. **Detection Rate:** Northern European >99%.

CLN3-related Neuronal Ceroid Lipofuscinosis - Gene: CLN3. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_001042432:2-16. **Detection Rate:** Northern European >99%.

CLN5-related Neuronal Ceroid Lipofuscinosis - Gene: CLN5. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_006493:1-4. **Detection Rate:** Northern European >99%.

CNGB3-related Achromatopsia - Gene: CNGB3. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_019098:1-18. **Detection Rate:** Northern European >99%.

Cohen Syndrome - Gene: VPS13B. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_017890:2-62. **Detection Rate:** Northern European 97%.

Congenital Disorder of Glycosylation Type Ia - Gene: PMM2. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000303:1-8. **Detection Rate:** Northern European >99%.

Congenital Disorder of Glycosylation Type Ib - Gene: MPI. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_002435:1-8. **Detection Rate:** Northern European >99%.

Congenital Finnish Nephrosis - Gene: NPHS1. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_004646:1-29. **Detection Rate:** Northern European >99%.

Costeff Optic Atrophy Syndrome - Gene: OPA3. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_025136:1-2. **Detection Rate:** Northern European >99%.

Cystic Fibrosis - Gene: CFTR. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000492:1-27. IVS8-5T allele analysis is only reported in the presence of the R117H mutation. **Detection Rate:** Northern European >99%.

Cystinosis - Gene: CTNS. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_004937:3-12. **Detection Rate:** Northern European >99%.

D-bifunctional Protein Deficiency - Gene: HSD17B4. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000414:1-24. **Detection Rate:** Northern European 98%.

Dihydropyrimidine Dehydrogenase Deficiency - Gene: DPYD. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000110:1-23. **Detection Rate:** Northern European 98%.

Factor XI Deficiency - Gene: F11. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000128:2-15. **Detection Rate:** Northern European >99%.

Familial Dysautonomia - Gene: IKBKAP. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_003640:2-37. **Detection Rate:** Northern European >99%.

Familial Mediterranean Fever - Gene: MEFV. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000243:1-10. **Detection Rate:** Northern European >99%.

Fanconi Anemia Type C - Gene: FANCC. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000136:2-15. **Detection Rate:** Northern European >99%.

FKTN-related Disorders - Gene: FKTN. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_001079802:3-11. **Detection Rate:** Northern European >99%.

Galactosemia - Gene: GALT. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000155:1-11. **Detection Rate:** Northern European >99%.

Gaucher Disease - Gene: GBA. Autosomal Recessive. Analysis of Homologous Regions. **Variants (10):** D409V, D448H, IVS2+1G>A, L444P, N370S, R463C, R463H, R496H, V394L, p.L29Afs*18. **Detection Rate:** Northern European 60%.

GJB2-related DFNB1 Nonsyndromic Hearing Loss and Deafness - Gene: GJB2. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_004004:1-2. **Detection Rate:** Northern European >99%.

Glutaric Acidemia Type 1 - Gene: GCDH. Autosomal Recessive. Sequencing with Copy Number Analysis. **Exons:** NM_000159:2-12. **Detection Rate:** Northern European >99%.

Glycogen Storage Disease Type Ia - Gene: G6PC. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000151:1-5. **Detection Rate:** Northern European >99%.

Glycogen Storage Disease Type Ib - Gene: SLC37A4. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_001164277:3-11. **Detection Rate:** Northern European >99%.

Glycogen Storage Disease Type III - Gene: AGL. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000642:2-34. **Detection Rate:** Northern European >99%.

Glycogen Storage Disease Type V - Gene: PYGM. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_005609:1-20. **Detection Rate:** Northern European >99%.

GRACILE Syndrome - Gene: BCS1L. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_004328:3-9. **Detection Rate:** Northern European >99%.

HADHA-related Disorders - Gene: HADHA. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000182:1-20. **Detection Rate:** Northern European >99%.

Hb Beta Chain-related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease) - Gene: HBB. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000518:1-3. **Detection Rate:** Northern European >99%.

Hereditary Fructose Intolerance - Gene: ALDOB. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000035:2-9. **Detection Rate:** Northern European >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMA3-related - Gene: LAMA3. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000227:1-38. **Detection Rate:** Northern European >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMB3-related - Gene: LAMB3. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000228:2-23. **Detection Rate:** Northern European >99%.

Herlitz Junctional Epidermolysis Bullosa, LAMC2-related - Gene: LAMC2. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_005562:1-23. **Detection Rate:** Northern European >99%.

Hexosaminidase A Deficiency (Including Tay-Sachs Disease) - Gene: HEXA. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000520:1-14. **Detection Rate:** Northern European >99%.

Homocystinuria Caused by Cystathionine Beta-synthase Deficiency - Gene: CBS. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000071:3-17. **Detection Rate:** Northern European >99%.

Hypophosphatasia, Autosomal Recessive - Gene: ALPL. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000478:2-12. **Detection Rate:** Northern European >99%.

Inclusion Body Myopathy 2 - Gene: GNE. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_001128227:1-12. **Detection Rate:** Northern European >99%.

Isovaleric Acidemia - Gene: IVD. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_002225:1-12. **Detection Rate:** Northern European >99%.

Joubert Syndrome 2 - Gene: TMEM216. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_001173990:1-5. **Detection Rate:** Northern European >99%.

Krabbe Disease - Gene: GALC. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000153:1-17. **Detection Rate:** Northern European >99%.

Lipoamide Dehydrogenase Deficiency - Gene: DLD. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000108:1-14. **Detection Rate:** Northern European >99%.

Maple Syrup Urine Disease Type 1B - Gene: BCKDHB. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_183050:1-10. **Detection Rate:** Northern European >99%.

Medium Chain Acyl-CoA Dehydrogenase Deficiency - Gene: ACADM. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000016:1-12. **Detection Rate:** Northern European >99%.

Megalencephalic Leukoencephalopathy with Subcortical Cysts - Gene: MLC1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_015166:2-12. **Detection Rate:** Northern European >99%.

Metachromatic Leukodystrophy - Gene: ARSA. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000487:1-8. **Detection Rate:** Northern European >99%.

Mucopolysaccharidosis IV - Gene: MCOLN1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_020533:1-14. **Detection Rate:** Northern European >99%.

Mucopolysaccharidosis Type I - Gene: IDUA. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000203:1-14. **Detection Rate:** Northern European >99%.

Muscle-eye-brain Disease - Gene: POMGNT1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_017739:2-22. **Detection Rate:** Northern European 96%.

NEB-related Nemaline Myopathy - Gene: NEB. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_001271208:3-80,117-183. **Detection Rate:** Northern European 92%.

Niemann-Pick Disease Type C - Gene: NPC1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000271:1-25. **Detection Rate:** Northern European >99%.

Niemann-Pick Disease, SMPD1-associated - Gene: SMPD1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000543:1-6. **Detection Rate:** Northern European >99%.

Nijmegen Breakage Syndrome - Gene: NBN. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_002485:1-16. **Detection Rate:** Northern European >99%.

Northern Epilepsy - Gene: CLN8. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_018941:2-3. **Detection Rate:** Northern European >99%.

PCDH15-related Disorders - Gene: PCDH15. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_033056:2-33. **Detection Rate:** Northern European 93%.

Pendred Syndrome - Gene: SLC26A4. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000441:2-21. **Detection Rate:** Northern European >99%.

PEX1-related Zellweger Syndrome Spectrum - Gene: PEX1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000466:1-24. **Detection Rate:** Northern European >99%.

Phenylalanine Hydroxylase Deficiency - Gene: PAH. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000277:1-13. **Detection Rate:** Northern European >99%.

PKHD1-related Autosomal Recessive Polycystic Kidney Disease - Gene: PKHD1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_138694:2-67. **Detection Rate:** Northern European >99%.

Polyglandular Autoimmune Syndrome Type 1 - Gene: AIRE. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000383:1-14. **Detection Rate:** Northern European >99%.

Pompe Disease - Gene: GAA. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000152:2-20. **Detection Rate:** Northern European 98%.

PPT1-related Neuronal Ceroid Lipofuscinosis - Gene: PPT1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000310:1-9. **Detection Rate:** Northern European >99%.

Primary Carnitine Deficiency - Gene: SLC22A5. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_003060:1-10. **Detection Rate:** Northern European >99%.

Primary Hyperoxaluria Type 1 - Gene: AGXT. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000030:1-11. **Detection Rate:** Northern European >99%.

Primary Hyperoxaluria Type 2 - Gene: GRHRP. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_012203:1-9. **Detection Rate:** Northern European >99%.

PROP1-related Combined Pituitary Hormone Deficiency - Gene: PROP1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_006261:1-3. **Detection Rate:** Northern European >99%.

Pseudocholinesterase Deficiency - Gene: BCHE. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000055:2-4. **Detection Rate:** Northern European >99%.

Pycnodysostosis - Gene: CTSK. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000396:2-8. **Detection Rate:** Northern European >99%.

Rhizomelic Chondrodysplasia Punctata Type 1 - Gene: PEX7. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000288:1-10. **Detection Rate:** Northern European >99%.

Salla Disease - Gene: SLC17A5. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_012434:1-11. **Detection Rate:** Northern European 98%.

Segawa Syndrome - Gene: TH. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000360:1-13. **Detection Rate:** Northern European >99%.

Short Chain Acyl-CoA Dehydrogenase Deficiency - Gene: ACADS. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000017:1-10. **Detection Rate:** Northern European >99%.

Sjogren-Larsson Syndrome - Gene: ALDH3A2. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000382:1-10. **Detection Rate:** Northern European 97%.

Smith-Lemli-Opitz Syndrome - Gene: DHCR7. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_001360:3-9. **Detection Rate:** Northern European >99%.

Spinal Muscular Atrophy - Gene: SMN1. Autosomal Recessive. Spinal Muscular Atrophy. **Variant (1):** SMN1 copy number. **Detection Rate:** Northern European 95%.

Steroid-resistant Nephrotic Syndrome - Gene: NPHS2. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_014625:1-8. **Detection Rate:** Northern European >99%.

Sulfate Transporter-related Osteochondrodysplasia - Gene: SLC26A2. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000112:2-3. **Detection Rate:** Northern European >99%.

TPP1-related Neuronal Ceroid Lipofuscinosis - Gene: TPP1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000391:1-13. **Detection Rate:** Northern European >99%.

Tyrosinemia Type I - Gene: FAH. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000137:1-14. **Detection Rate:** Northern European >99%.

Usher Syndrome Type 3 - Gene: CLRN1. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_174878:1-3. **Detection Rate:** Northern European >99%.

Very Long Chain Acyl-CoA Dehydrogenase Deficiency - Gene: ACADVL. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000018:1-20. **Detection Rate:** Northern European >99%.

Wilson Disease - Gene: ATP7B. Autosomal Recessive. Sequencing with Copy Number Analysis. Exons: NM_000053:1-21. **Detection Rate:** Northern European >99%.

Risk Calculations

Below are the risk calculations for all conditions tested. Since negative results do not completely rule out the possibility of being a carrier, the **residual risk** represents the patient's post-test likelihood of being a carrier and the **reproductive risk** represents the likelihood the patient's future children could inherit each disease. These risks are inherent to all carrier screening tests, may vary by ethnicity, are predicated on a negative family history and are present even after a negative test result. Inaccurate reporting of ethnicity may cause errors in risk calculation. The reproductive risk presented is based on a hypothetical pairing with a partner of the same ethnic group.

Disease	DONOR 10177 Residual Risk	Reproductive Risk
21-hydroxylase-deficient Congenital Adrenal Hyperplasia	1 in 1,400	1 in 310,000
ABCC8-related Hyperinsulinism	1 in 11,000	< 1 in 1,000,000
Alkaptonuria	1 in 6,800	< 1 in 1,000,000
Alpha Thalassemia	Alpha globin status: aa/aa.	Not calculated
Alpha-1 Antitrypsin Deficiency	1 in 2,700	1 in 300,000
Alpha-mannosidosis	1 in 35,000	< 1 in 1,000,000
Alpha-sarcoglycanopathy	1 in 45,000	< 1 in 1,000,000
Andermann Syndrome	< 1 in 50,000	< 1 in 1,000,000
ARSACS	< 1 in 44,000	< 1 in 1,000,000
Aspartylglycosaminuria	< 1 in 50,000	< 1 in 1,000,000
Ataxia with Vitamin E Deficiency	< 1 in 50,000	< 1 in 1,000,000
Ataxia-telangiectasia	1 in 8,200	< 1 in 1,000,000
Bardet-Biedl Syndrome, BBS1-related	1 in 16,000	< 1 in 1,000,000
Bardet-Biedl Syndrome, BBS10-related	1 in 16,000	< 1 in 1,000,000
Beta-sarcoglycanopathy	< 1 in 50,000	< 1 in 1,000,000
Biotinidase Deficiency	1 in 13,000	1 in 670,000
Bloom Syndrome	< 1 in 50,000	< 1 in 1,000,000
Canavan Disease	< 1 in 31,000	< 1 in 1,000,000
Carnitine Palmitoyltransferase IA Deficiency	< 1 in 50,000	< 1 in 1,000,000
Carnitine Palmitoyltransferase II Deficiency	< 1 in 50,000	< 1 in 1,000,000
Cartilage-hair Hypoplasia	< 1 in 50,000	< 1 in 1,000,000
Citrullinemia Type 1	1 in 12,000	< 1 in 1,000,000
CLN3-related Neuronal Ceroid Lipofuscinosis	1 in 22,000	< 1 in 1,000,000
CLN5-related Neuronal Ceroid Lipofuscinosis	< 1 in 50,000	< 1 in 1,000,000
CNGB3-related Achromatopsia	1 in 11,000	< 1 in 1,000,000
Cohen Syndrome	< 1 in 15,000	< 1 in 1,000,000
Congenital Disorder of Glycosylation Type Ia	1 in 16,000	< 1 in 1,000,000
Congenital Disorder of Glycosylation Type Ib	< 1 in 50,000	< 1 in 1,000,000
Congenital Finnish Nephrosis	< 1 in 50,000	< 1 in 1,000,000
Costeff Optic Atrophy Syndrome	< 1 in 50,000	< 1 in 1,000,000
Cystic Fibrosis	1 in 2,700	1 in 290,000
Cystinosis	1 in 22,000	< 1 in 1,000,000
D-bifunctional Protein Deficiency	1 in 9,000	< 1 in 1,000,000
Dihydropyrimidine Dehydrogenase Deficiency	< 1 in 29,000	< 1 in 1,000,000
Factor XI Deficiency	< 1 in 50,000	< 1 in 1,000,000
Familial Dysautonomia	< 1 in 50,000	< 1 in 1,000,000
Familial Mediterranean Fever	< 1 in 50,000	< 1 in 1,000,000
Fanconi Anemia Type C	1 in 16,000	< 1 in 1,000,000
FKTN-related Disorders	< 1 in 50,000	< 1 in 1,000,000
Galactosemia	1 in 8,600	< 1 in 1,000,000
Gaucher Disease	1 in 280	1 in 120,000
GJB2-related DFNB1 Nonsyndromic Hearing Loss and Deafness	1 in 3,200	1 in 420,000
Glutaric Acidemia Type 1	1 in 10,000	< 1 in 1,000,000
Glycogen Storage Disease Type Ia	1 in 18,000	< 1 in 1,000,000
Glycogen Storage Disease Type Ib	1 in 35,000	< 1 in 1,000,000
Glycogen Storage Disease Type III	1 in 16,000	< 1 in 1,000,000
Glycogen Storage Disease Type V	1 in 16,000	< 1 in 1,000,000
GRACILE Syndrome	< 1 in 50,000	< 1 in 1,000,000
HADHA-related Disorders	1 in 15,000	< 1 in 1,000,000

Disease	DONOR 10177 Residual Risk	Reproductive Risk
Hb Beta Chain-related Hemoglobinopathy (Including Beta Thalassemia and Sickle Cell Disease)	1 in 5,000	1 in 990,000
Hereditary Fructose Intolerance	1 in 8,000	< 1 in 1,000,000
Herlitz Junctional Epidermolysis Bullosa, LAMA3-related	< 1 in 50,000	< 1 in 1,000,000
Herlitz Junctional Epidermolysis Bullosa, LAMB3-related	< 1 in 50,000	< 1 in 1,000,000
Herlitz Junctional Epidermolysis Bullosa, LAMC2-related	< 1 in 50,000	< 1 in 1,000,000
Hexosaminidase A Deficiency (Including Tay-Sachs Disease)	1 in 30,000	< 1 in 1,000,000
Homocystinuria Caused by Cystathionine Beta-synthase Deficiency	1 in 25,000	< 1 in 1,000,000
Hypophosphatasia, Autosomal Recessive	1 in 16,000	< 1 in 1,000,000
Inclusion Body Myopathy 2	< 1 in 50,000	< 1 in 1,000,000
Isovaleric Acidemia	1 in 25,000	< 1 in 1,000,000
Joubert Syndrome 2	< 1 in 50,000	< 1 in 1,000,000
Krabbe Disease	1 in 15,000	< 1 in 1,000,000
Lipoamide Dehydrogenase Deficiency	< 1 in 50,000	< 1 in 1,000,000
Maple Syrup Urine Disease Type 1B	1 in 25,000	< 1 in 1,000,000
Medium Chain Acyl-CoA Dehydrogenase Deficiency	1 in 5,900	< 1 in 1,000,000
Megalencephalic Leukoencephalopathy with Subcortical Cysts	< 1 in 50,000	< 1 in 1,000,000
Metachromatic Leukodystrophy	1 in 20,000	< 1 in 1,000,000
Mucopolidosis IV	< 1 in 50,000	< 1 in 1,000,000
Mucopolysaccharidosis Type I	1 in 16,000	< 1 in 1,000,000
Muscle-eye-brain Disease	< 1 in 12,000	< 1 in 1,000,000
NEB-related Nemaline Myopathy	< 1 in 6,700	< 1 in 1,000,000
Niemann-Pick Disease Type C	1 in 19,000	< 1 in 1,000,000
Niemann-Pick Disease, SMPD1-associated	1 in 25,000	< 1 in 1,000,000
Nijmegen Breakage Syndrome	1 in 16,000	< 1 in 1,000,000
Northern Epilepsy	< 1 in 50,000	< 1 in 1,000,000
PCDH15-related Disorders	1 in 5,300	< 1 in 1,000,000
Pendred Syndrome	1 in 7,000	< 1 in 1,000,000
PEX1-related Zellweger Syndrome Spectrum	1 in 11,000	< 1 in 1,000,000
Phenylalanine Hydroxylase Deficiency	1 in 5,000	1 in 990,000
PKHD1-related Autosomal Recessive Polycystic Kidney Disease	1 in 6,100	< 1 in 1,000,000
Polyglandular Autoimmune Syndrome Type 1	1 in 14,000	< 1 in 1,000,000
Pompe Disease	1 in 6,300	< 1 in 1,000,000
PPT1-related Neuronal Ceroid Lipofuscinosis	< 1 in 50,000	< 1 in 1,000,000
Primary Carnitine Deficiency	< 1 in 50,000	< 1 in 1,000,000
Primary Hyperoxaluria Type 1	1 in 35,000	< 1 in 1,000,000
Primary Hyperoxaluria Type 2	< 1 in 50,000	< 1 in 1,000,000
PROP1-related Combined Pituitary Hormone Deficiency	1 in 11,000	< 1 in 1,000,000
Pseudocholinesterase Deficiency (Mild Condition)	1 in 2,700	1 in 300,000
Pycnodysostosis	< 1 in 50,000	< 1 in 1,000,000
Rhizomelic Chondrodysplasia Punctata Type 1	1 in 16,000	< 1 in 1,000,000
Salla Disease	< 1 in 30,000	< 1 in 1,000,000
Segawa Syndrome	< 1 in 50,000	< 1 in 1,000,000
Short Chain Acyl-CoA Dehydrogenase Deficiency	1 in 16,000	< 1 in 1,000,000
Sjogren-Larsson Syndrome	1 in 9,100	< 1 in 1,000,000
Smith-Lemli-Opitz Syndrome	1 in 4,900	1 in 970,000
Spinal Muscular Atrophy	Negative for g.27134T>G SNP SMN1: 2 copies 1 in 770	1 in 110,000
Steroid-resistant Nephrotic Syndrome	1 in 40,000	< 1 in 1,000,000
Sulfate Transporter-related Osteochondrodysplasia	1 in 11,000	< 1 in 1,000,000
TPP1-related Neuronal Ceroid Lipofuscinosis	1 in 30,000	< 1 in 1,000,000
Tyrosinemia Type I	1 in 17,000	< 1 in 1,000,000
Usher Syndrome Type 3	< 1 in 50,000	< 1 in 1,000,000
Very Long Chain Acyl-CoA Dehydrogenase Deficiency	1 in 8,800	< 1 in 1,000,000
Wilson Disease	1 in 8,600	< 1 in 1,000,000

Patient Information

Name: Donor 10177
 Date of Birth: [REDACTED]
 Sema4 ID: 20131174CS
 Client ID: SEATSB-S410177
 Indication: Carrier Testing

Specimen Information

Specimen Type: Blood
 Date Collected: 07/31/2020
 Date Received: 08/01/2020
 Final Report: 08/23/2020

Referring Provider

Jeffrey Olliffe, M.D.
 Seattle Sperm Bank
 4915 25th Avenue NE
 Seattle, WA, 98105
 Fax: 206-466-4696

Custom Carrier Screen (ECS)

Number of genes tested: 1

SUMMARY OF RESULTS AND RECOMMENDATIONS

Negative

Negative for all genes tested: DHDDS

To view a full list of genes and diseases tested
 please see Table 1 in this report

AR=Autosomal recessive; XL=X-linked

Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.

Test description

This patient was tested for the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please view the Table of Residual Risks Based on Ethnicity at the end of this report or at go.sema4.com/residualrisk for gene transcripts, sequencing exceptions, specific detection rates, and residual risk estimates after a negative screening result. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.

Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit go.sema4.com/residualrisk

Table 1: List of genes and diseases tested with detailed results

Disease	Gene	Inheritance Pattern	Status	Detailed Summary
⊖ Negative				
Retinitis Pigmentosa 59	DHDDS	AR	Reduced Risk (see table below)	

AR=Autosomal recessive; XL=X-linked

Table 2: Residual Risk by ethnicity for negative results

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Retinitis Pigmentosa 59 (AR) NM_024887.3	DHDDS	Ashkenazi Jewish	1 in 100	99%	1 in 9,900	99%
		European (Non-Finnish)	1 in 6008	99%	1 in 601,000	
		Native American	1 in 4223	99%	1 in 422,000	
		Worldwide	1 in 2009	99%	1 in 201,000	

Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).
 ‡ Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD Fg GJB2 GJB1 GLA* and *MEFV* gene testing only).
 † Please note that *GJB2* testing includes testing for the two upstream deletions, del(*GJB6-D13S1830*) and del(*GJB6-D13S1854*) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).
 AR Autosomal recessive N/A Not available XL X-linked

Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™ QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. This sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping

assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Copy Number Variant Analysis (Analytical Detection Rate >95%)

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom array CGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

Exon Array (Confirmation method) (Accuracy >99%)

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

Quantitative PCR (Confirmation method) (Accuracy >99%)

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard $\Delta\Delta C_t$ formula.

Long-Range PCR (Analytical Detection Rate >99%)

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with these sequencing output to analyze the results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

Sanger Sequencing (Confirmation method) (Accuracy >99%)

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med* 2013 15:482-3.

Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015 May;17(5):405-24

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
Patient Name: Donor 10177 Date of Birth: ██████████ Reference #: SEATSB-S410177 Indication: Carrier Testing Test Type: Custom Carrier Screen (ECS)	Specimen Type: Blood Lab #: 19141030CS Date Collected: 3/22/2019 Date Received: 3/23/2019 Final Report: 4/6/2019	Jeffrey Olliffe, M.D. Seattle Sperm Bank 4915 25th Avenue NE Suite 204W Seattle, WA 98105 Fax: 206-466-4696

Results

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *ACAD9*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *ACAD9* gene which is associated with mitochondrial complex I deficiency (*ACAD9*-related) was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient: Donor 10177

DOB: [REDACTED]

Lab #: 19141030CS

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Mitochondrial Complex I Deficiency (ACAD9-Related) (AR) NM_014049.4	ACAD9	African	1 in 784	86%	1 in 5,600	99%
		Ashkenazi Jewish	1 in 1239	99%	1 in 124,000	
		East Asian	1 in 2252	75%	1 in 9,100	
		Finnish	1 in 2094	83%	1 in 12,200	
		Caucasian	1 in 309	83%	1 in 1,900	
		Latino	1 in 741	90%	1 in 7,700	
		South Asian	1 in 810	68%	1 in 2,500	
		Worldwide	1 in 472	84%	1 in 2,900	

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient: Donor 10177

DOB: [REDACTED]

Lab #: 19141030CS

Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)

PCR amplification using Asuragen, Inc. AmplideX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping (Analytical Detection Rate >99%)

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA[®] probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*3+80T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*3+80T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*3+80T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*3+80T>G variant allele; these will be reported if confirmed to be located in *SMN1* using locus-specific Sanger primers

MLPA for Gaucher disease (*GBA*), cystic fibrosis (*CFTR*), and non-syndromic hearing loss (*GJB2/GJB6*) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854).

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DOB: [REDACTED]

Lab #: 19141030CS

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Copy Number Variant Analysis (Analytical Detection Rate >95%)

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

Exon Array (Confirmation method) (Accuracy >99%)

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

Quantitative PCR (Confirmation method) (Accuracy >99%)

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard $\Delta\Delta C_t$ formula.

Long-Range PCR (Analytical Detection Rate >99%)

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2*, *HBA1* and *HBA2* and *GBA*. The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with the sequencing output to analyze the results. For *CYP21A2*, a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to

Patient: Donor 10177

DOB: [REDACTED]

Lab #: 19141030CS

determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Residual Risk Calculations

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

Sanger Sequencing (Confirmation method) (Accuracy >99%)

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥98%)

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.

Fragile X syndrome:

Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

Spinal Muscular Atrophy:

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med.* 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

Duchenne Muscular Dystrophy:

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat.* 2009 30:1657-66.

Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.

Patient: Donor 10177

DOB: [REDACTED]

Lab #: 19141030CS



RESULTS RECIPIENT
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 Phone: (206) 588-1484
 Fax: (206) 466-4696
 NPI: 1306838271
 Report Date: 08/05/2020

MALE
DONOR 10177
 DONOR 10177
 DOB: [REDACTED]
 Ethnicity: Northern European
 Sample Type: EDTA Blood
 Date of Collection: 07/28/2020
 Date Received: 07/30/2020
 Date Tested: 08/04/2020
 Barcode: 11004512662774
 Accession ID: CSLE3HCK36RH24M
 Indication: Egg or sperm donor

FEMALE
 N/A

Foresight® Carrier Screen

NEGATIVE

ABOUT THIS TEST

The **Myriad Foresight Carrier Screen** utilizes sequencing, maximizing coverage across all DNA regions tested, to help you learn about your chance to have a child with a genetic disease.

RESULTS SUMMARY

Risk Details	DONOR 10177	Partner
Panel Information	Foresight Carrier Screen Reflex Panel (F5) (1 condition tested)	N/A
All conditions tested A complete list of all conditions tested can be found on page 3.	<input type="checkbox"/> NEGATIVE No disease-causing mutations were detected.	N/A

CLINICAL NOTES

- None

NEXT STEPS

- If necessary, patients can discuss residual risks with their physician or a genetic counselor.

Methods and Limitations

DONOR 10177 [Foresight Carrier Screen]: Targeted genotyping (DTS v3.1).

Targeted genotyping

Targeted DNA mutation analysis is used to determine the genotypes of the listed variants in the Conditions Tested section of the report.

Limitations

In an unknown number of cases, nearby genetic variants may interfere with mutation detection. Other possible sources of diagnostic error include sample mix-up, trace contamination, bone marrow transplantation, blood transfusions and technical errors. This test is designed to detect and report germline alterations. While somatic variants present at low levels may be detected, these may not be reported. If more than one variant is detected in a gene, additional studies may be necessary to determine if those variants lie on the same chromosome or different chromosomes. This test is not designed to detect sex chromosome copy number variations. If present, sex chromosome abnormalities may significantly reduce test sensitivity for X-linked conditions. Residual and reproductive risks provided assume a normal karyotype. Risks for individuals with abnormal karyotypes may be different. The test does not fully address all inherited forms of intellectual disability, birth defects and genetic disease. A family history of any of these conditions may warrant additional evaluation. Furthermore, not all mutations will be identified in the genes analyzed and additional testing may be beneficial for some patients. For example, individuals of African, Southeast Asian, and Mediterranean ancestry are at increased risk for being carriers for hemoglobinopathies, which can be identified by CBC and hemoglobin electrophoresis or HPLC (*ACOG Practice Bulletin No. 78. Obstet. Gynecol. 2007;109:229-37*).

This test was developed and its performance characteristics determined by Myriad Women's Health, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's evaluation. CLIA Number: **#05D1102604**.

Resources

GENOME CONNECT | <http://www.genomeconnect.org>

Patients can share their reports via research registries such as Genome Connect, an online research registry working to build the knowledge base about genetics and health. Genome Connect provides patients, physicians, and researchers an opportunity to share genetic information to support the study of the impact of genetic variation on health conditions.

SENIOR LABORATORY DIRECTOR



Jack Ji, PhD, FACMG

Report content approved by Jack Ji, PhD, FACMG on Aug 5, 2020



RESULTS RECIPIENT
SEATTLE SPERM BANK
Attn: Jeffrey Olliffe
NPI: 1306838271
Report Date: 08/05/2020

MALE
DONOR 10177
DOB: [REDACTED]
Ethnicity: Northern European
Barcode: 11004512662774

FEMALE
N/A

Conditions Tested

Factor V Leiden Thrombophilia - Gene: F5. Autosomal Recessive. Targeted genotyping. Variant (1): c.1601G>A. Detection Rate: Unknown due to rarity of disease.



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FEMALE
N/A

Risk Calculations

Below are the risk calculations for all conditions tested. Since negative results do not completely rule out the possibility of being a carrier, the **residual risk** represents the patient's post-test likelihood of being a carrier and the **reproductive risk** represents the likelihood the patient's future children could inherit each disease. These risks are inherent to all carrier screening tests, may vary by ethnicity, are predicated on a negative family history and are present even after a negative test result. Inaccurate reporting of ethnicity may cause errors in risk calculation. The reproductive risk presented is based on a hypothetical pairing with a partner of the same ethnic group.

Disease	DONOR 10177 Residual Risk	Reproductive Risk
Factor V Leiden Thrombophilia (Mild Condition)	Not calculated	Not calculated